PITFIELD (R.L.)

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A NEW METHOD OF STAINING FLAGELLA.

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LOEFFLER'S method of staining flagella is probably the one most commonly and generally employed. This consists in treating the bacteria to be stained with a mordant made up of tannic acid and ferrous sulphate, and then staining the bacteria with a solution of an anilin color in water.

I have devised a method which is simple, and, in my hands, much more reliable and easier of execution. It is as simple as the staining of bacteria with ordinary carbol-fuchsin, and I have stained over fifty preparations of flagellated micro-organisms, each time demonstrating the flagella most satisfactorily.

The method consists in the use of but a single solution, which is at once mordant and stain. The solution should be made in two parts, which are filtered and mixed.

A.

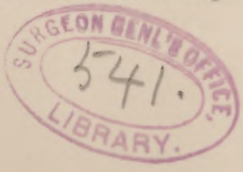
Saturated aqueous solution of alum . .	10 c.cm.
Saturated alcoholic solution of gentian-violet	1 c.cm.

B.

Tannic acid	1 gm.
Distilled water	10 c.cm.

The solutions should be made with cold water, and immediately after mixing the stain is ready for use.

The cover-slip is to be carefully cleaned, the grease being burned off in a flame, and after it has cooled the bacteria are spread upon it, well diluted in water, care being taken to exclude culture-medium. After the preparation has been thoroughly dried in the air it should be held over the flame with the fingers, as Loeff-



ler has directed. Afterward the stain is gradually poured on the slip and heated gently, bringing the fluid almost to a boil; the slip covered with the hot stain should then be laid aside for one minute, then washed in water and mounted.

Upon examination, the bacteria, both isolated and in clumps, will, if motile, be found to have the flagella clearly and delicately defined. In the middle of the cover-slip, as well as around the edges, the bacteria will be found equally well stained; the clumps being surounded by a zone of delicate fringing flagella, each being well stained and distinctly outlined from its fellows.

If a clean preparation is desired, the stain, after mixing, may be filtered, but I have found that the most reliable method is to use the unfiltered stain. In the case of the former a clear field is produced without the detritus, etc., precipitated on the glass around the microorganisms; and all the flagella are stained, but not so distinctly as with the unfiltered solution.

If the filtered stain is used, a second stain of anilin-water containing gentian-violet had better be used, which should be applied but a moment and then washed off, thus leaving a clean field, showing only the bacteria lightly stained, with their flagella still more lightly colored.

In examining the different bacteria, I have found that the bacillus of typhoid fever, the colon-bacillus, the cholera-bacillus, and the bacillus of hog-cholera, each stained well by this method, and without the addition of any acid or alkali to the mordant, such as Loeffler uses.

The bacillus of typhoid fever showed the flagella most beautifully, and there seemed one flagellum to each cell that stained more deeply than the others and appeared larger and stronger.

As to the keeping qualities of the stain I have not fully ascertained, but presumably it should be mixed daily to yield the best results.